

Early View

Original research article

The Palestinian primary ciliary dyskinesia population: first results of the diagnostic, and genetic spectrum

Nisreen Rumman, Mahmoud R. Fassad, Corine Driessens, Patricia Goggin, Nader Abdelrahman, Adel Adwan, Mutaz Albakri, Jagrati Chopra, Regan Doherty, Bishara Fashho, Grace M. Freke, Abdallah Hasaballah, Claire L Jackson, Mai A. Mohamed, Reda Abu Nema, Mitali P. Patel, Reuben J Pengelly, Ahmad Qaaqour, Bruna Rubbo, N. Simon Thomas, James Thompson, Woolf T. Walker, Gabrielle Wheway, Hannah M. Mitchison, Jane S. Lucas

Please cite this article as: Rumman N, Fassad MR, Driessens C, *et al.* The Palestinian primary ciliary dyskinesia population: first results of the diagnostic, and genetic spectrum. *ERJ Open Res* 2023; in press (<https://doi.org/10.1183/23120541.00714-2022>).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

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Funding and support:

NR was the recipient of a European Respiratory Society Fellowship (STRTF 2014-6816). AAIR Charity (Charity number: 1129698) funded whole exome sequencing; Circassia loaned two Niox Mino analysers for this study. The National PCD Diagnostic Service at University Hospital Southampton (UHS) is commissioned and funded by NHS England. Some authors are participants of BEAT-PCD: Better Evidence to Advance Therapeutic options for PCD (COST Action BM 1407; European Respiratory Society (ERS) Clinical Research Collaboration). BEAT-PCD COST Action funded JSL to visit NR to support the new PCD Service and development of this cohort in Palestine. NR was funded on an Academy of Medical Sciences Daniel Turnberg Travel Fellowship to visit HMM for completion of the genetic data. HMM acknowledges funding from Great Ormond Street Children's Charity, NIHR Biomedical Research Centre at Great Ormond Street Hospital, the British Council Newton-Mosharafa Fund and Ministry of Higher Education in Egypt. Some authors participate in European Reference Network for Rare Respiratory Diseases (ERN-LUNG) - Project ID No 739546. JSL chaired the European Respiratory Society Task Force for the development of a practice guideline for diagnosis of PCD (ERS TF-2014-04) and ERS Task Force for Nasal nitric oxide measurement in children for the diagnosis of primary ciliary dyskinesia: a technical standard. MRF is supported by a Wellcome Trust Collaborative Award in Science (210585/Z/18/Z).

Roles and responsibilities

NR and JSL planned the diagnostic pathway and development of a PCD cohort, and CLJ taught NR to take and process diagnostic samples during an ERS Fellowship visit to UoS. NR collected clinical data, nasal brushings and DNA samples; NR holds the ethical approvals for the Palestinian Cohort. NR performed all the nasal NO testing. Electron microscopy was analysed by PG, RD and JT. Genetic samples were analysed by MF, GMF, MM, MPP, RP, GW and HMM. The data analysis plan was developed by JSL and BR. Genetic data analysis was performed by MF, NST, GW and HMM. Data cleaning was led by CD and JC. Clinical data analyses were undertaken by CD and JSL. BF conducted spirometry. NA, AA, RAN, AQ, AH, MA, and AH identify and refer patients for PCD diagnostic testing to NR. HMM and JSL take responsibility for the data and analyses.

Abstract (235 words)

Diagnostic testing for primary ciliary dyskinesia (PCD) started in 2013 in Palestine. We aimed to describe the diagnostic, genetic, and clinical spectrum of the Palestinian PCD population.

Individuals with symptoms suggestive of PCD were opportunistically considered for diagnostic testing: nasal nitric oxide (nNO) measurement, transmission electron microscopy (TEM), and/or PCD genetic panel or whole exome testing. Clinical characteristics of those with a positive diagnosis were collected close to testing including FEV₁ GLI z-scores, and BMI z-scores.

Sixty-eight individuals had a definite positive PCD diagnosis, 31 confirmed by genetic and TEM results, 23 by TEM results alone, and 14 by genetic variants alone. Forty-five individuals from 40 families had seventeen clinically actionable variants, and 4 had variants of unknown significance in 14 PCD-genes. *CCDC39*, *DNAH11*, and *DNAAF11* were the most commonly mutated genes. 100% of variants were homozygous. Patients had median age of 11.2 years at diagnosis, were highly consanguineous (93%) and 100% of Arabic descent. Clinical features included persistent wet cough (99%), neonatal respiratory distress (84%), and situs inversus (43%). Lung function at diagnosis was already impaired (FEV₁ z-score median -1.90 (-5.0 to 1.32)) and growth was mostly within the normal range (z-score mean= -0.36 (-3.03 to 2.57). 19% individuals had finger clubbing.

Despite limited local resources, detailed geno- and phenotyping forms the basis of one of the largest national PCD populations globally. There was notable familial homozygosity within the context of significant population heterogeneity.

Key words: cohort profile, Primary Ciliary Dyskinesia, rare diseases, epidemiology

Introduction

Primary ciliary dyskinesia (PCD) is a rare heterogeneous disease that can be caused by genetic mutations in over 50 different motile cilia-related genes(1-3). The associated functional and structural defects of cilia impair mucociliary clearance, causing persistent upper and lower respiratory disease, including daily wet cough and recurrent chest infections, chronic serous otitis media (glue ear), and persistent rhinosinusitis(4). Pulmonary symptoms often start in the neonatal period and continue throughout life, almost invariably leading to bronchiectasis. Involvement of embryonic nodal cilia causes situs anomalies in approximately 50% of individuals, some with associated congenital heart defects(5). PCD is estimated to affect about 1 in 7500 people in Europe but is more common in populations where consanguineous marriage is common(6, 7).

Palestine has a highly consanguineous population, and many patients in its paediatric pulmonology service have symptoms suggestive of PCD. PCD diagnostic testing is complex and requires specialist equipment and personnel, hence innovative approaches are needed in countries with fewer resources(8). According to European Respiratory Society and American Thoracic Society guidelines, a positive diagnosis can only be made in individuals with a supportive clinical history and either confirmatory 'hallmark' ultrastructural abnormalities detected by transmission electron microscopy (TEM), or pathogenic mutations in PCD genes that show a consistent inheritance pattern (respective to the recessive, X-linked and dominant subtypes of PCD)(9, 10). The diagnosis is likely, but not proven, in individuals with a persistent wet cough from infancy, with either situs anomalies or a low nasal nitric oxide (nNO) measurement (11-13).

While great progress has been made in the past decade to understand molecular disease mechanisms and improve diagnosis and treatment, PCD management is still hampered by a lack of fundamental data on epidemiology and clinical course in different settings. To date, a handful of national cohorts have been described, and these are almost exclusively based in well-resourced healthcare settings(14). Most published data is cross-sectional, and longitudinal cohorts are now required to extend the knowledge base for PCD.

In this study, we aimed to demonstrate that accurate diagnoses are possible in a resource-limited country, by collaboration with specialist centres. We report the diagnostic findings including the genetic profile and recurrent disease-causing variants in the Palestinian PCD population. We additionally present the spectrum of clinical phenotypes, as well as disease severity close to the time of diagnosis.

Methods

Ethical approval

Ethical approval was provided by Al-Quds University Research Ethics Committee Palestine (Ref No: 31/REC/2018), London Bloomsbury Research Ethics Committee, UK (08/H0713/82) and Southampton and South-West Hants Research Ethics Committee (06/Q1702/10) with University of Southampton (ERGO 53155) UK. Participants or parents gave informed consent.

Study design

Patients were evaluated and opportunistically identified for PCD diagnostic testing by the only physician testing patients in Palestine (NR), in collaboration with TEM and genetics experts at University of Southampton (UoS) and University College London (UCL). Participants were reported in the study if they had a positive PCD diagnosis based on diagnostic TEM and/ or genetic testing (9). Data was prospectively captured using clinic records and retrospectively added to a study dataset. Key variables for this manuscript are diagnostic results (TEM and nNO), genotype, and cross-sectional clinical characteristics at time of diagnosis including lung function (FEV₁), nutritional status, and demographics (June 2013 to December 2020).

Clinical characteristics

The clinical history, spirometry and anthropometry data were recorded by one clinician (NR) at the time of diagnostic testing. Data were retrospectively transferred to a data capture sheet (Excel) with predefined variables. Where necessary, the patient's family was re-contacted following clinic to clarify demographics or symptoms. Spirometry was measured using Smartpftlab™ (Medical Equipment Europe GmbH, Hammelburg, Germany) according to ATS/ERS guidelines (15), and FEV₁ and FVC z-scores were calculated using ERS Global Lung Index (GLI) reference values (16). BMI was calculated from clinic measurements of height and weight, and z-scores were calculated using the World Health Organisation (WHO) BMI 5-19 years reference values (<https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age>). In the absence of a suitable reference for adults we estimated the z-score for adults assuming they were 19 years old (all adults with BMI measurements in the study were aged ≤48 years).

Diagnosis

Diagnostic testing was directed by clinical indication and available resources. Nasal NO measurement was conducted as a screening tool from March 2016, and if low then a nasal brushing sample was sent for TEM. Genetic testing was limited by funding, and priorities changed over time (e.g. initially prioritising patients with an abnormal TEM(17), and once confidence at identifying the clinical

phenotype and measuring nNO was reached, patients with normal TEM but highly likely clinical picture and low nNO were included. On occasions the order of tests differed; for example, if nNO analysis was not possible on the day of nasal brushing it was sometimes measured later. Diagnostic results were transferred to a data capture sheet (Excel) with predefined variables and codes. Many families had several individuals with symptoms, but because of limited resources, in most cases only one person from each family was tested.

Nasal nitric oxide: Patients who were developmentally able (e.g. aged >5 years) were screened using a portable electrochemical nNO analyser (Niox Mino; March 2016 – April 2019; Niox Vero September 2019 – December 2020). Measurements used tidal breathing with pursed lips method whenever possible and the open mouth method in younger children. The measurements were taken when patients were free of acute infection, at a sampling flow of 0.3 L/min. A low nNO is not in itself confirmatory of PCD(9) and symptomatic individuals with levels <77nL/min were considered highly likely to have PCD, and were not included in this study unless genetics or TEM confirmed the diagnosis.

Transmission Electron Microscopy: For patients with a strong clinical suspicion based on signs and symptoms (13), ciliated respiratory epithelium was sampled for TEM by brushing the nasal mucosa using a cytology brush. Samples were chemically fixed in glutaraldehyde and sent to UoS for quantitative analysis. Using a Tecnai Spirit 12 TEM at 26,500x minimum magnification, 100-300 cilia in transverse section per sample were scored for assessment of axonemal structure, and representative images captured. Using BEAT-PCD criteria, a positive diagnosis of PCD was confirmed by identification of outer dynein arm defect, inner and outer dynein arm defects, or microtubular disorganisation with inner dynein arm defect (17). Defects which did not fulfil the criteria for a positive diagnosis (e.g. transposition defect; mis-localisation of basal bodies with few or no cilia) were recorded, but these participants were not considered to have a definite diagnosis unless they had genetic confirmation (9).

Genetic testing and analysis: Blood samples were obtained from probands, their parents and their affected and unaffected siblings where possible. DNA was isolated from peripheral blood lymphocytes by standard procedures, then subject to next generation sequencing either using a targeted gene panel as previously described(18) or whole exome sequencing (WES). For WES, exome libraries (Agilent SureSelect Human All Exon V6 kit, Agilent Technologies) were paired-end 150-bp sequenced on an Illumina HiSeq2500 by Novogene (UK) Co Ltd. Bioinformatic analyses were performed as described previously for the gene panel(18). Fastq files from WES were aligned to the human reference genome (GRCh38/hg38) using BWA-MEM2(19). Variant calling was performed

according to the GATK 4.1.4.1 best practice pipeline (20) and variant annotation was done using Annovar (21). Variant prioritization and filtration were performed as previously described (18). All variants were reported according to HGVS recommendations(22) and mapped to GRCh38. Variant pathogenicity annotation was performed according to ACMG/AMP variant interpretation guidelines (23). Variant classification information was also extracted from DECIPHER and ClinVar, a database providing aggregated variant interpretation data collected from clinical and research testing laboratories, expert panels and other groups (24, 25). Sanger sequencing was used to confirm all variants prioritized using NGS and assure correct segregation within family members. In three families, targeted Sanger sequencing was also used on a candidate variant basis for genetic diagnosis to identify recurrent *CCDC39* and *DNAAF11* mutations in this population shared with patients with similar TEM findings (**Supplementary table 1**).

Data management and statistical analyses

Data were cleaned and coded in Excel using predefined coding, and then imported into SAS9.4 (SAS Institute, Cary NC) to be formatted and analysed. Descriptive statistics were performed in SAS9.4 using the PROC FREQ, PROC UNIVARIATE, and PROC SQL functions. Continuous data were described as mean with standard deviation (SD) or median with interquartile range (IQR), and categorical data were described as proportions. Data were tidied using Tidyverse packages. For visualization, plots were created with the ggplot2 package using the ggplot function with more colours added using RColorBrewer package in R-studio 3.6 (R-Studio team, Boston MA). Genotype-phenotype clustering was visualised with a Circos plot using the RCircos package. Genetic data from 13 patients were included in a previous publication (20).

Results

Four hundred and sixty-four patients with a clinical history suggestive of PCD were opportunistically investigated. Nasal NO was measured in 350, TEM analysed in 183 and genetic screening in 82 (**Supplementary Figure 1**), following which 68 had a positive diagnosis according to ERS Guidelines (9). Twenty-three individuals had a positive diagnosis based on TEM alone, 14 on pathogenic biallelic mutations alone (only recessive forms of PCD were identified), with 31 having both genetic and TEM diagnostic confirmation.

Of 34 individuals with 'hallmark' diagnostic TEM who proceeded to genetic testing, 31 (91%) had causative variants in known PCD genes; no variants in known PCD genes were found in the other 3 but putative candidate genes with variants of interest are currently being investigated. Of those with normal TEM, 21 proceeded to genetic testing of whom 11 had a genetic cause found (52%) (*DNAH11*, *DRC1*, *RSPH4* and *RSPH9*). The genetic and confirmatory familial segregation analysis data are summarised in **Supplementary Table 1**.

Palestinian PCD population clinical characteristics (n=68)

The mean age at diagnosis was 10.0 years (range 3 months- 40 years), 39.7% were female and 42.6% had situs inversus (**Table 1**). Individuals were almost all from consanguineous backgrounds (92.6%), and many had symptomatic family members. The reporting of symptoms including neonatal respiratory distress (83.8%), persistent wet cough (98.5%), persistent rhinosinusitis (94.1%), and middle ear disease (73.5%) were similar to previous reports (4), but finger clubbing (19.1%) was reportedly higher than expected (0/65; unpublished Southampton Children's PCD Clinic, UK). Whilst the vast majority of patients reported a combination of several symptoms (36% had chronic cough, rhinosinusitis, neonatal respiratory distress and situs inversus; 45% had chronic cough, rhinosinusitis and neonatal respiratory distress with normal situs), several patients reported fewer symptoms highlighting the heterogeneity of the syndrome (**Figure 1**).

nNO was <77nl/min in 100% of tested individuals with a positive diagnosis. There was considerable variability of FEV₁, FVC and BMI z-scores, but median values for lung function were already below average around the time of diagnosis (**Table 1**).

Genetic causes of PCD in the Palestinian population

Genetic screening was performed on 82 patients from 63 families. This identified a total of 17 different causal variants and an additional 4 variants of unknown significance (VUS) in 14 known PCD genes, according to ACMG-AMP variant classification guidelines (23) (**Table 2, Figure 2, Supplementary Table 1**). Of the 4 variants classed as VUS there were several lines of evidence to support pathogenicity but all fell below the 'likely pathogenic' threshold, except for one well reported variant *CCDC103* c.104G>C; p.(Arg35Pro), which is likely pathogenic if PP4 evidence is applied (phenotype/biochemical/IF specificity) (**Table 2**). Eight of the pathogenic/ likely pathogenic variants identified in this study were specific to the Palestinian population, not having been reported before in ClinVar (**Table 2**). A genetic diagnosis in agreement with confirmation through proband sequencing and familial segregation analysis to identify biallelic variants meeting ACMG-AMP pathogenic/likely pathogenic criteria (23) resulted in a definitive genetic cause being found in a total of 45 patients: 39 affected individuals from 35 families; additionally, VUS in four known PCD genes consistent with TEM defects were identified in a further 6 individuals from 5 families (**Table 2**). One variant, *CCDC40* c.48A>G carried in a family with 4 affected children, differed between its ClinVar score ('likely benign') and ACMG score of 'likely pathogenic', as it is predicted to create a synonymous early missense change, p.(Gly16Gly), but ACMG predicts a likely splicing defect (**Table 2**). Overall, the genetic screening analysis yielded a positive diagnosis in 45 patients from 40 families, providing a positive genetic diagnosis in 62% families and 55% of patients, who all carried homozygous variants in known PCD genes. The other 37 patients screened did not carry indicative variants in known PCD genes, but a number of variants in candidate ciliary genes were identified that are being further investigated.

TEM findings correlated with genotype (**Table 3, Supplementary Table 1**). A remarkable 100% of genetic diagnoses involved homozygous variants, yet the homozygous variants were found in 12 different genes (14 genes including VUS results, **Figure 2**). This reflects a high degree of genetic heterogeneity, alongside family allelic homogeneity reflecting likely shared parental ancestry within families. It is also notable that seven variants were recurrent within the population i.e. detected in two or more families, suggesting a shared ancestral mutation/founder effect: *CCDC39* c.1871_1872del; p.(Ile624Lysfs*3), *CCNO* c.381+5G>C, *DNAAF4* c.384_390del; p.(Tyr128*), *DNAAF11* c.436G>C; p.(Asp146His), *DRC1* c.1521_1524del; p.(Glu508Alafs*4), and *RSPH9* c.800-802del; p.(Lys268del) as well as a *DNAH11* VUS p.(Met188Thr) (**Figure 2, Table 2, supplementary table 1**).

This hypothesis is supported by previous reports showing that three of these recurrent variants were carried in PCD patients of Arabic Bedouin and Palestinian origin (26-31) (**Table 2**).

For the two most commonly occurring mutations, 9 families carrying the same homozygous *CCDC39* c.1871_1872del variants and 5 carrying homozygous *DNAAF11* c.436G>C, together account for 35% of overall diagnosis alone. The variants *DNAAF4* c.384_390del and *RSPH9* c.800-802del found in 6 families account for another 15%. These four variants alone therefore cause significant levels of disease amongst the tested Palestinian families and highlight that in less resourced settings, identification of commonly occurring disease alleles can allow for highly targeted genetic diagnosis. As illustrated in this study, it was possible to use allele-specific PCR for diagnostics in three families carrying *CCDC39* c.1871_1872del and *DNAAF11*c.436G>C mutations (**Supplementary table 1**).

Despite commonly recurrent mutations, the number of genetically confirmed patients was insufficient to identify meaningful genotype-phenotype correlations amongst the population (**Supplementary figure 2**). Even individuals with the same variant, and some from the same family, have differing clinical presentations.

Discussion

This is the first report of PCD diagnostic findings, including genetic variants, within the Palestinian population. In eight years, we successfully established a diagnostic process, resulting in a large clinical population of 68 individuals with confirmed PCD from Palestine. A further 57 are highly likely to have the diagnosis based on situs inversus with sinopulmonary symptoms and/ or low nasal nitric oxide levels, and many more symptomatic family members will now undergo targeted screening. The data includes clinical manifestations, genotype, and axoneme ultrastructure. This work highlights the importance of international collaboration for rare diseases like PCD, particularly where the diagnosis is complex and requires expertise and equipment that is only available in specialist centres. Networks such as BEATPCD and ERN-LUNG support the diagnosis and management of PCD by supporting local physicians.

As expected, the clinical characteristics were similar to those previously described including neonatal respiratory distress, persistent wet cough from infancy and middle ear disease(4, 13). Interestingly, when we first started diagnosing patients, we had a predominance of patients with laterality defects, but the incidence of situs inversus in the clinic population is now only 42.6%, presumably reflecting the improved detection by other features.

The genotyping revealed a high diagnostic rate and a fascinating genetic architecture for this population. Remarkably, families from this small geographical isolate show extensive genetic heterogeneity, since 12 different PCD genes were found to carry 17 different causal mutations amongst the affected families. A further 4 VUS of unproven diagnostic status, except for one that may be causal if PP4 applied from the ACMG classification system, were detected in 4 of the known PCD genes. Just as striking however, is the finding that all genetic diagnoses involved homozygous variants in the affected individuals, with the parents in each individual family both being heterozygotes carrying a single copy of the same mutant allele. There was one case of a family with an affected father carrying the same homozygous variants as their affected child, but this relates to a VUS of less certain causation (*RSPH9* c.760del). We found a number of recurrent mutations affecting two or more families, carried in homozygous state in 26 PCD families. Most prominent of the recurrent mutations were *CCDC39* c.1871_1872del and *DNAAF11* c.436G>C. This reflects common ancestry and direct consanguinity amongst Palestinian marriages, with cousin marriages being common (32). Notably, homozygous variants were also found in the children of the few parents who were not knowingly related, reflecting a high population endogamy.

The literature on PCD from the Arab countries has mostly been limited to a few cases reports and case series(33). A study of 33 families from Egypt reported genetic heterogeneity with variants in 13 genes(34), but although the responsible genes were similar to the ones described in this study, the variants were unique to either Palestinian or Egyptian families. A study from United Arab Emirates identified a number of unique deleterious variants, but also a large number of variants of unknown significance in PCD-related genes(35). In a cohort from Tunisia (34 families), *CCDC39* was the most common gene (44.1%) similar to our population, followed by *DNAH5* (11.8%), *HYDIN* (8.8%) and *TTC25* (5.9%)(36). In Saudi Arabia the most commonly mutated gene was *RSPH9* among 18 cases, which included the *RSPH9* c.800-802del mutation also reported here (37).

A study from Israel included predominantly Arab individuals, and reported mutations in *DNAH11* (38) to be the most common cause of PCD. A study of five children from two unrelated consanguineous Palestinian families revealed novel mutations in *DNAAF11/LRRC6* (c.436G>C), which also affected families in this study(28).

There are four main providers of health care in Palestine; the Ministry of Health responsible for the public health system, the United Nations Relief and Works Agency for Palestinian Refugees (UNRWA), non-governmental (NGO) and civil society organizations (like the Palestinian Red Crescent), and the private health sector. Although there have been significant health care improvements in recent years, the system still lacks sufficient infrastructure to serve the growing

population. According to the Palestinian Central Bureau of Statistics (PCBS) (www.pcbs.gov.ps), in 2018 the average number of doctors per 1000 population was 2.09 and hospital beds 1.33 per 1000 population which is considerably less than most developed countries. The health care system is closely connected to, and affected by the social, economic, and political situations. Therefore, it faces challenges due to many years of restrictions and blockade. In 2017, PCBS reported that 29.2% of Palestinians were living below the poverty level, about four times higher in Gaza (53%) than West Bank (13.9%) (www.pcbs.gov.ps). This makes it challenging for many Palestinians to access quality health care. The diagnostic testing conducted during this study was dependent on a number of small research grants with support from collaborators in UK. Moving forward, the PCD service in Palestine is not funded by the public health system, therefore patients will have to either pay out of pocket or purchase an expensive private insurance to cover the costs of clinic visits and tests.

In addition, the blockade on the West Bank and Gaza and the restrictions on free movement make it difficult for many Palestinians to have immediate access to health care. Patients with chronic medical conditions like PCD, who should ideally have regular monitoring, often seek medical attention only when they are ill, and cannot afford routine monitoring such as sputum microbiology, imaging, and pulmonary function testing. These challenges were significant obstacles for a standardised approach to PCD diagnostic testing in Palestine in this study. The choice and order of the tests depended on the resources available at the time and the access to clinics. For example, in Gaza, it was easier for local physicians to ship blood samples for genetic testing rather than patients travelling from Gaza to the West Bank for nasal NO and ciliary biopsy.

Moreover, the lack of a specialised multidisciplinary PCD centre in Palestine is another barrier to providing PCD care for patients. Diagnostic services are fractioned and potentially inconsistent due to nasal NO measurements and nasal brushings being conducted at four different locations (Jerusalem, Bethlehem, Hebron and Ramallah).

Identifying the causative mutations in the Palestinian population can allow targeted mutation testing in future cases that could be traced back to one of the known families in this study. Such testing can be done locally at a cheap cost thus saving resources and avoiding many of the other cumbersome tests if a diagnosis could be reached. However, such an approach should be interpreted with caution since it may be good to “rule in” but not “rule out” PCD. In the future, it may even be possible to develop a population-specific mutation panel that could be utilised for a newborn screening program.

In summary, we report the first detailed study of the genetics and clinical features of PCD in the Palestinian population. The cohort of individuals with PCD was rapidly formed within an isolated

population with limited diagnostic resources, and continues to grow. There are a large number of families and individuals with symptoms who are still awaiting investigation and it is likely that the prevalence of PCD in Palestine will be higher than the reported in international literature. We have highlighted the significant genetic heterogeneity and the remarkable finding of 100% homozygous variants.

Table 1: Demographics and clinical characteristics of participants in the PCD cohort, i.e. those with definite diagnosis of PCD based on TEM and/or genetic testing. n=68 unless otherwise stated

Clinical variables

Age at diagnosis (years)	Median 10.0; range 3months-40.0
Female	27 (39.7%)
Situs inversus	29 (42.6%)
Consanguinity n=60	65 (92.6%)
Congenital heart disease*n=67	8 (11.9%)
Neonatal respiratory symptoms n=67	57 (85.1%)
Persistent wet cough	57 (83.8%)
Persistent rhinosinusitis	64 (94.1%)
Conductive hearing loss and /or otitis media with effusion (glue ear)	50 (73.5%)
Finger clubbing n=67	13 (19.4%)
nNO (nL/min) n=36	Median=14.5; Range 5 to 62.5
FEV ₁ z-score n=28	Median= -1.90 (-5.0 - 1.32)
FVC z-score n=28	Median= -1.72 (-5.64 - 0.57)
BMI z-score n=28	Median=-0.36 (-3.03 - 2.57)

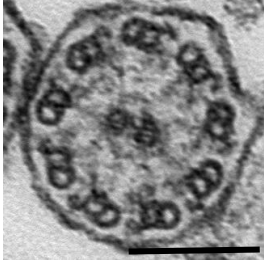
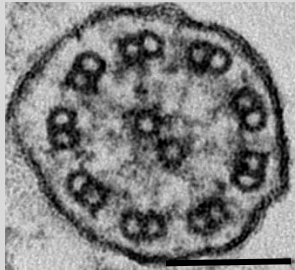
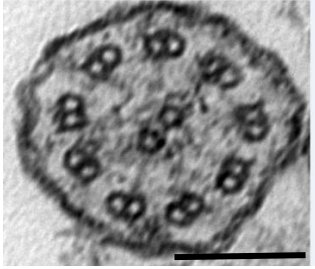
**Atrial Septal Defect (ASD) n=2; Right Isomerism with complex cardiac defects n=1; Situs ambiguous with complex cardiac defects n=1; Subaortic membrane n=1; Ventricular Septal Defect n=3;*

Table 2. Pathogenicity classifications for all identified variants in known PCD genes. Study ID indicates family number and each family has a single affected child with a positive genetic diagnosis, except where ‘aff’ is shown, indicating that there is more than one affected child in the family (number of affected individuals is shown in these cases). Family 0-13 is the only family with an affected parent in addition to affected children. DECIPHER, ACMG-AMP and ClinVar classifications indicate likelihood that variants cause protein loss-of-function, transcript nonsense mediated decay (NMD), in addition to showing their predicted pathogenic, likely pathogenic or VUS status.

Study ID	Gene	RefSeq transcript	Variant (all homozygous)	Genomic location (GRCh38)	Exon/Total	LOF	NMD (Decipher)	ACMG/AMP evidences	ACMG/AMP classification	ClinVar classification	Recorded in previous publications/similar population
0-1, 0-2, 0-8, 0-9, 0-11, 0-20, 0-21, 0-25, 0-31	CCDC39	NM_181426.1	c.1871_1872del; p.(Ile624Lysfs*3)	3: 180641995_180641996del	13/20	Yes	Yes	PVS1_very strong PM2_moderate PM3_moderate	Pathogenic	Pathogenic	1 case from Saudi exome project (26)
0-35	CCDC39	NM_181426.1	c.2190del; p.(Glu731Asnfs*31)	3: 180619334del	16/20	Yes	Yes	PVS1_very strong PM2_moderate PM3_moderate	Pathogenic	Pathogenic	Algerian/Tunisian founder effect (27, 39)
0-6 (4 aff)	CCDC40	NM_017950.3	c.48A>G; p.(Gly16Gly)	17: 80038141A>G	2/20	No	N/A	PM2_moderate PM3_supporting PP1_strong	Likely Pathogenic (may create splice enhancer)	Likely benign	No
0-36, 0-39	CCNO	NM_021147.5	c.381+5G>C	5: 55233138C>G	1/3	No	No	PM2_moderate PM3_moderate PP1_moderate PP3_supporting PP4_supporting	Likely Pathogenic	Absent	No
0-16, 0-30, 0-40	DNAAF4 (DYX1C1)	NM_130810.3	c.384_390del; p.(Tyr128*)	15: 55491138_55491144del	5/10	Yes	Yes	PVS1_very strong PM2_moderate PM3_moderate	Pathogenic	Likely Pathogenic	No
0-4, 0-15, 0-18, 0-26, 0-42	DNAAF11 (LRRC6)	NM_012472.4	c.436G>C; p.(Asp146His)	8: 132632957C>G	5/12	No	N/A	PS3_strong PM3_moderate PP1_strong	Pathogenic	Pathogenic	2 unrelated Palestinian families (28, 40)
0-23 (2 aff)	DNAH5	NM_001369.2	c.10050G>A; p.(Trp3350*)	5: 13766027C>T	59/79	Yes	Yes	PVS1_very strong PM2_moderate PM3_supporting	Pathogenic	Absent	No
0-24	DNAH11	NM_001277115.1	c.6727C>T; p.(Arg2243*)	7: 21710596C>T	41/82	Yes	Yes	PVS1_very strong PM3_moderate	Pathogenic	Pathogenic/Likely Pathogenic	(41)
0-7	DNAH11	NM_001277115.1	c.12646G>T; p.(Glu4216*)	7: 21892563G>T	77/82	Yes	Yes	PVS1_very strong PM2_moderate PM3_moderate	Pathogenic	Absent	No
0-22	DNAH11	NM_001277115.1	c.13240dup; p.(Thr4414Asnfs*34)	7: 21900057dup	81/82	Yes	Yes	PVS1_very strong PM2_moderate PM3_moderate	Pathogenic	Pathogenic	No
0-28	DNAH11	NM_001277115.1	c.13436_13440dup; p.(Tyr4481Leufs*7)	7: 21901139_21901143dup	82/82	Yes	No	PVS1_moderate PM2_moderate PM3_moderate	Likely Pathogenic	Absent	No
0-14, 0-19	DRC1	NM_145038.5	c.1521_1524del; p.(Glu508Alafs*4)	2: 26450009_26450012del	12/17	Yes	Yes	PVS1_strong PM2_moderate PM3_moderate	Likely Pathogenic	Absent	No
0-5	ODAD3 (CCDC151)	NM_145045.4	c.850C>T; p.(Gln284*)	19: 11426257G>A	7/13	Yes	Yes	PVS1_strong PM2_moderate PM3_supporting	Likely Pathogenic	Absent	No
0-29	RSPH4A	NM_001010892.3	c.367del; p.(Pro123Leufs*44)	6: 116616991del	1/6	Yes	Yes	PVS1_very strong PM2_moderate PM3_supporting	Pathogenic	Absent	No
0-37	RSPH4A	NM_001010892.3	c.72G>A; p.(Trp24*)	6: 116616695G>A	1/6	Yes	Yes	PVS1_very strong PM2_moderate PM3_supporting	Pathogenic	Absent	No
0-27, 0-32, 0-33	RSPH9	NM_152732.4	c.800_802del; p.(Lys268del)	6: 43670922_43670924del	5/5	No	N/A	PS3_strong PM2_supporting PM3_moderate	Pathogenic	Pathogenic	2 Bedouin families; 1 Saudi family (29, 30, 42)

								PM4_supporting PP1_strong			
0-34	SPAG1	NM_172218.3	c.742C>T; p.(Arg248*)	8: 100187160C>T	8/19	Yes	Yes	PVS1_very strong PM2_moderate PM3_supporting PP1_supporting	Pathogenic	Absent	No
0-3	CCDC103	NM_213607.3	c.104G>C; p.(Arg35Pro)	17: 44901102G>C	2/4	No	N/A	PM2_moderate PM3_moderate PP3_supporting PP4_supporting	VUS (likely pathogenic)	VUS	No
0-10, 0-12	DNAH11	NM_001277115.1	c.563T>C; p.Met188Thr	7:21558869T>C	3/82	No	N/A	PM2_moderate PM3_moderate PP1_supporting	VUS	Absent	No
0-17	DNAL1	NM_031427.4	c.285_287del; p.(Glu97del)	14: 73687283_73687285del	6/8	No	No	PM2_moderate PM3_supporting PM4_supporting	VUS	VUS	No
0-13 (**aff + aff father)	RSPH9	NM_152732.4	c.760del; p.(Arg254Alafs*76)	6: 43670878del	5/5	Yes	No	PVS1_moderate PM2_moderate PM3_supporting	VUS	Absent	No

Table 3: Diagnostic outcomes in the Palestinian PCD population. Nasal nitric oxide (nNO) and PCD-genes with pathogenic mutations are reported according to TEM findings. Two patients with a genetic diagnosis did not have TEM conducted (*RSPH9* and *DNAH5*). All scale bars = 200 nm

Normal electron microscopy with genetic cause found N=12 	Median nNO (n=9): 17.5 nl/min (6.0 – 62.5) Genetic cause: <i>DNAH11 (incl VUS)</i> 6 <i>RSPH9</i> 3 <i>DRC1</i> 2 <i>RSPH4A</i> 1	
Outer dynein arm defect n= 13 	Median nNO (n=9): 10 nL/min (5 – 26) Genetic cause: <i>ODAD3 (CCDC151)</i> 1 <i>DNAAF4</i> 1 <i>DNAH5</i> 1 <i>DNAAF11</i> 1 <i>SPAG1</i> 1 <i>DNAL1 (VUS)</i> 1 No genetic cause found/ genetic testing not conducted 7	
Outer dynein arm & Inner dynein arm defect N=13 	Median (nNO n=7): 9.0 nL/min (5.5 – 10) Genetic cause: <i>DNAAF4</i> 2 <i>CCDC103 (VUS)</i> 1 <i>DNAAF11</i> 4 No genetic cause found/ genetic testing not conducted 6	

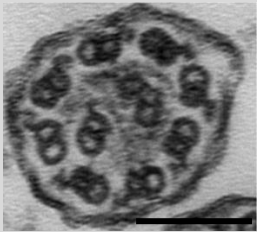
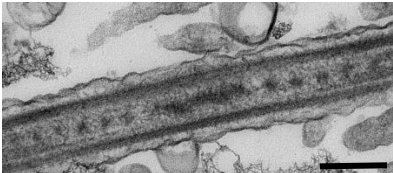
Microtubular disorganisation with inner dynein arm defect n=20 	Median nNO (n=8): 29.5 nL/min (10.5 – 56.4)	
	Genetic cause:	
	<i>CCDC39</i>	10
	<i>CCDC40</i>	2
Central pair defect +/- transposition defect n=4 	Median nNO (n=3) 3.3 nL/min (1.5 – 5.4)	
	Genetic cause:	
	<i>RSPH4A</i>	1
	<i>RSPH9 (incl VUS)</i>	2
Mislocalisation of basal bodies with few or no cilia n=3	nNO (n=1): 23.5 nL/min	
	Genetic cause:	
	<i>CCNO</i>	2
	No genetic cause found/ genetic testing not conducted (following guidelines these are not definitely positive and are not in the cohort)	1

Figure Legends

Figure 1: Venn diagram representing the variability of symptoms characteristic of PCD (n=67; insufficient data for one member of cohort): situs inversus, neonatal respiratory distress, chronic wet cough, and rhinosinusitis.

Figure 2: Range of homozygous gene variants identified in 45 genetically diagnosed Palestine PCD patients. Per gene, each different block represents a different variant, as listed in Table 2. Numbers within the blocks indicate the number of patients per mutation.

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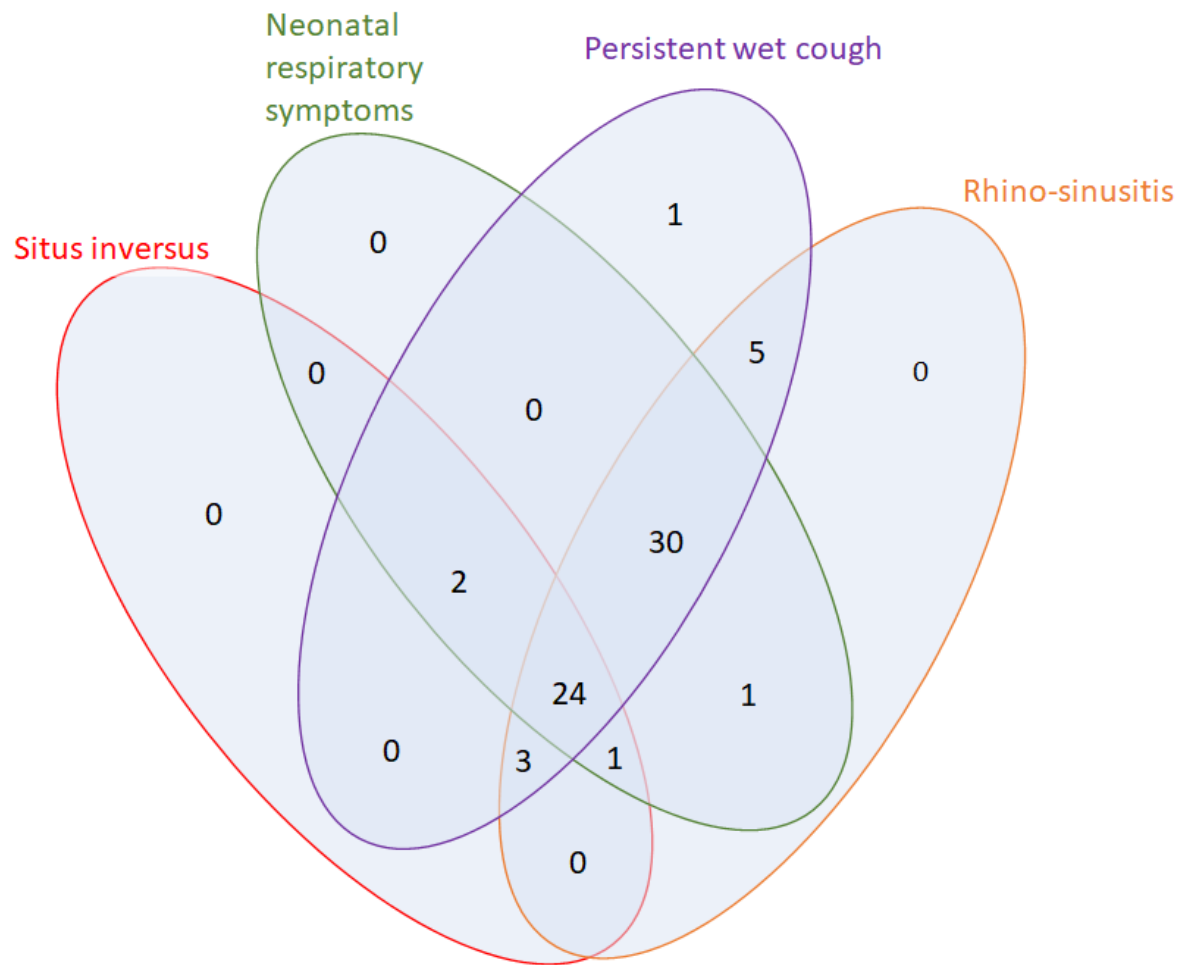


Figure 1: Venn diagram representing the variability of symptoms characteristic of PCD (n=67; insufficient data for one member of cohort): situs inversus, neonatal respiratory distress, chronic wet cough, and rhinosinusitis.

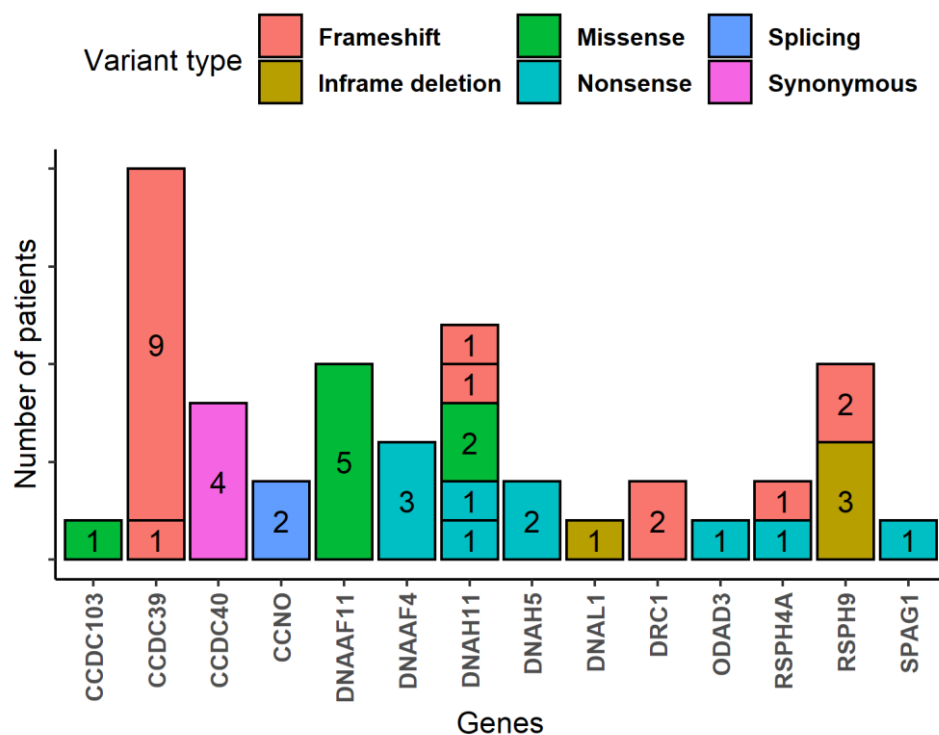



Figure 2: Range of homozygous gene variants identified in 45 genetically diagnosed Palestine PCD patients. Per gene, each different block represents a different variant, as listed in Table 2. Numbers within the blocks indicate the number of patients per mutation.

Title: The Palestinian primary ciliary dyskinesia population: first results of the diagnostic, and genetic spectrum

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Supplementary table and Figures

Supplementary table 1. Gene variants, familial genetics and screening method for 45 individuals (from 40 families) positively genetically diagnosed in the study. Panel, targeted gene panel; WES, whole exome sequencing (Agilent SureSelect Human All Exon V6); MTD, microtubular disorganisation; IDA, inner dynein arm defect; ODA, outer dynein arm defect; CC, central pair complex defect; T, transposition of peripheral microtubules. [§]A total 51 individuals carried causal variants, with 6 individuals listed at the bottom carrying homozygous VUS (variant of unknown significance) not regarded as diagnostic without further evidence.

Supplementary Figure 1 Outcomes of test results for people with a clinical suspicion of PCD. The order of tests was usually as suggested by the flow diagram, but on occasions the order was different e.g. nNO sometimes followed TEM.  = PCD confirmed according to ERS guidelines.

Supplementary Figure 2: Genetic diagnoses associated with different PCD disease features in Palestine PCD patients

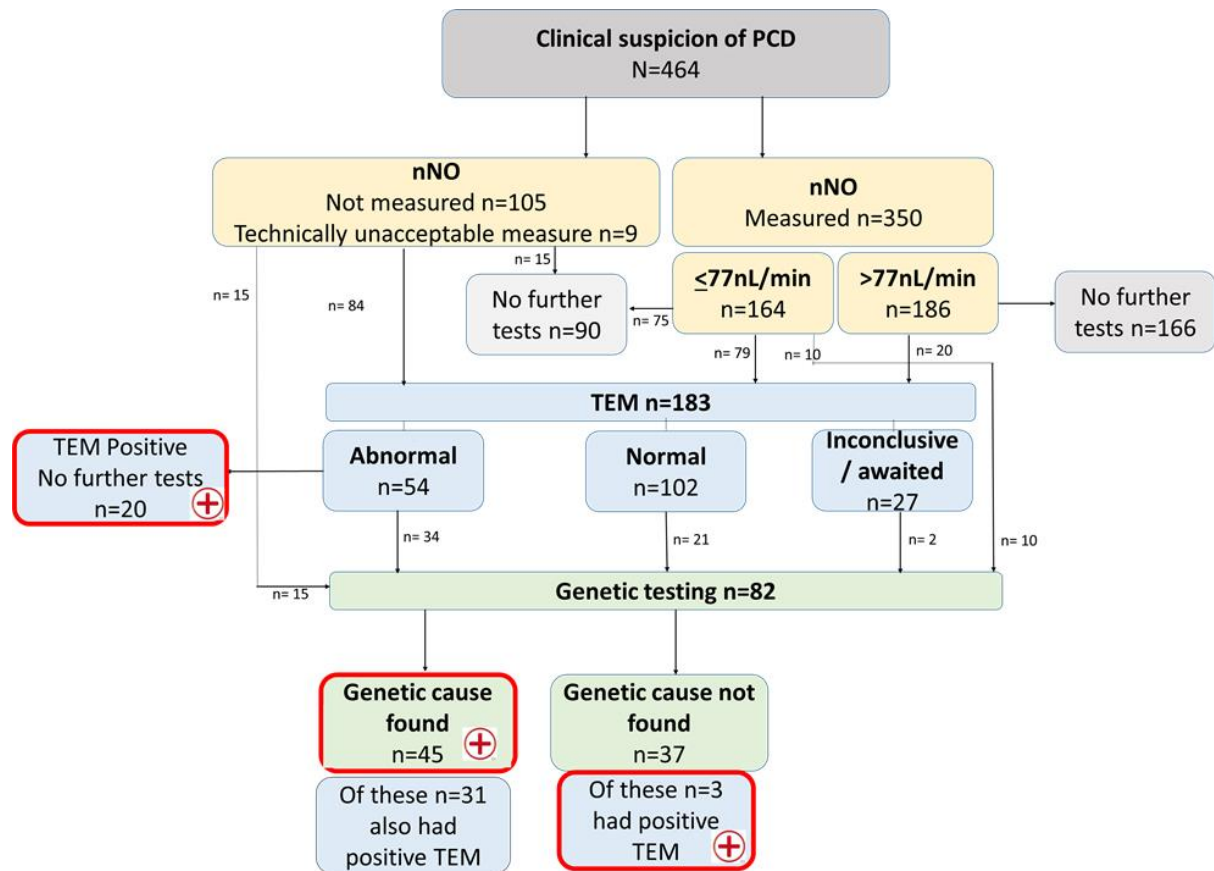
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Family Study ID	Gene	Variant (all homozygous) [§]	No. affected children with homozygous mutations	Parents available and consistent for segregation	Consistent segregation in # additional siblings	Sequencing method	TEM consistent
0-1	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	4	Panel	MTD+IDA
0-2	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	5	Panel	MTD
0-8	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	1	Panel	MTD
0-9	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	1	Panel	MTD+IDA
0-11	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	5	Panel	MTD+IDA
0-20	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	0	Panel	MTD+IDA
0-21	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	2	Panel	MTD+IDA
0-25	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	1	Allele-specific PCR	MTD+IDA
0-31	<i>CCDC39</i>	c.1871_1872del; p.(Ile624Lysfs*3)	1	Mat and Pat	0	Allele-specific PCR	MTD+IDA
0-35	<i>CCDC39</i>	c.2190del; p.(Glu731Asnfs*31)	1	Mat and Pat	2	WES	MTD+IDA

0-6	<i>CCDC40</i>	c.48A>G; p.(Gly16Gly)	4	Mat and Pat	2	Panel	MTD+IDA
0-36	<i>CCNO</i>	c.381+5G>C	1	Mat and Pat	1	WES	Oligocilia
0-39	<i>CCNO</i>	c.381+5G>C	1	Mat and Pat	5	WES	Oligocilia
0-16	<i>DNAAF4</i> (<i>DYX1C1</i>)	c.384_390del; p.(Tyr128*)	1	Mat only	0	Panel	ODA+IDA
0-30	<i>DNAAF4</i> (<i>DYX1C1</i>)	c.384_390del; p.(Tyr128*)	1	Mat and Pat	4	WES	ODA
0-40	<i>DNAAF4</i> (<i>DYX1C1</i>)	c.384_390del; p.(Tyr128*)	1	Mat and Pat	3	WES	ODA+IDA
0-4	<i>DNAAF11</i> (<i>LRRC6</i>)	c.436G>C; p.(Asp146His)	1	Mat and Pat	2	Panel	ODA+IDA
0-15	<i>DNAAF11</i> (<i>LRRC6</i>)	c.436G>C; p.(Asp146His)	1	Mat only	4	Panel	ODA+IDA
0-18	<i>DNAAF11</i> (<i>LRRC6</i>)	c.436G>C; p.(Asp146His)	1	Mat only	0	Panel	ODA+IDA
0-26	<i>DNAAF11</i> (<i>LRRC6</i>)	c.436G>C; p.(Asp146His)	1	Mat and Pat	3	WES	ODA
0-42	<i>DNAAF11</i> (<i>LRRC6</i>)	c.436G>C; p.(Asp146His)	1	Mat and Pat	0	Allele- specific PCR	ODA+IDA
0-23	<i>DNAH5</i>	c.10050G>A; p.(Trp3350*)	2	Mat and Pat	2	Panel	ODA
0-24	<i>DNAH11</i>	c.6727C>T; p.(Arg2243*)	1	Mat and Pat	3	Panel	Normal
0-7	<i>DNAH11</i>	c.12646G>T; p.(Glu4216*)	1	Mat and Pat	1	Panel	Normal
0-22	<i>DNAH11</i>	c.13240dup; p.(Thr4414Asnfs*34)	1	Mat and Pat	4	Panel	Normal
0-28	<i>DNAH11</i>	c.13436_13440dup; p.(Tyr4481Leufs*7)	1	Mat and Pat	5	WES	Normal
0-14	<i>DRC1</i>	c.1521_1524del;	1	Mat and Pat	3	WES	Normal

		p.(Glu508Alafs*4)					
0-19	<i>DRC1</i>	c.1521_1524del; p.(Glu508Alafs*4)	1	Mat and Pat	2	WES	Normal
0-5	<i>ODAD3</i> (<i>CCDC151</i>)	c.850C>T; p.(Gln284*)	1	Mat and Pat	2	Panel	ODA
0-29	<i>RSPH4A</i>	c.367del; p.(Pro123Leufs*44)	1	Mat and Pat	4	WES	CC+T
0-37	<i>RSPH4A</i>	c.72G>A; p.(Trp24*)	1	No parents	0	WES	CC+T
0-27	<i>RSPH9</i>	c.800-802del; p.(Lys268del)	1	Mat and Pat	2	WES	CC+T
0-32	<i>RSPH9</i>	c.800-802del; p.(Lys268del)	1	No parents	0	WES	CC
0-33	<i>RSPH9</i>	c.800-802del; p.(Lys268del)	1	Mat only	0	WES	CC+T
0-34	<i>SPAG1</i>	c.742C>T; p.(Arg248*)	1	Mat and Pat	4	WES	ODA
0-3	<i>CCDC103</i>	c.104G>C; p.(Arg35Pro) (VUS)	1	Mat and Pat	2	Panel	ODA+IDA
0-10	<i>DNAH11</i>	c.563T>C; p.Met188Thr (VUS)	1	Mat and Pat	0	Panel	Normal
0-12	<i>DNAH11</i>	c.563T>C; p.Met188Thr (VUS)	1	Mat only	0	Panel	Insufficient
0-17	<i>DNAL1</i>	c.285_287del; p.(Glu97del) (VUS)	1	Mat and Pat	5	WES	ODA
0-13	<i>RSPH9</i>	c.760del; p.(Arg254Alafs*76) (VUS)	1 (+1, as father also affected)	Mat and Pat (father also homozygous)	0	Panel	CC+T

Supplementary Figure 1 Outcomes of test results for people with a clinical suspicion of PCD. The order of tests was usually as suggested by the flow diagram, but on occasions the order was different e.g. nNO sometimes followed TEM. ⊕ = PCD confirmed according to ERS guidelines.



Supplementary Figure 2: Genetic diagnoses associated with different PCD disease features in Palestine PCD patients.

